fragment thereof is effected after stroke onset in the human subject.

REMARKS

Claims 1-13 and 16-20, 22-24, and 27 are currently pending in the above-identified application. By this Amendment, applicants have hereinabove canceled claims 3-6, 8, 16, 20 and 21. Claims 2, 7, 9-13, 17-19, 22-24 and 27 have been amended to more particularly point out and distinctly claim the subject matter which the applicants regard as the invention. Support for the amendment to claim 1 can be found in the specification at page 6, lines 2-8. Support for the amendment to claim 2 can be found in the specification at page 6, lines 10-12. Support for the amendment to claim 7 can be found in the specification at page 12, lines 24-26. Support for the amendment to claims 9-11 can be found in the specification at page 13, lines 7-18. Support for the amendment to claims 12 and 13 can be found in the specification at page 13, lines 20-25. Support for the amendment to claim 17 can be found in the specification at page 14, lines 19-33. Support for the amendment to claim 19 can be found in the specification at page 15, lines 8-10. Support for the amendment to claims 22-24 can be found in the specification at page 15, lines 18-27. Support for the amendment to claim 27 can be found in the specification at page 6, lines 2-8 and page 10, lines 12-17. Support for new claim 28 can be found in the specification at page 14, lines 19-32. Support for new claim 29 can be found in the specification at page 6, lines 21 and 22. Support for new claim 30 can be found in the specification at page 6, lines 24-26. Support for new claims 31-35 can be found in the specification at page 13, lines 27-35 and page 14, lines 1 and 2. Support for new claims 36-38 can be found in the

specification at page 13, lines 7-18. Applicants maintain that this amendment raises no issue of new matter.

A copy of the marked-up version of the amended claim is attached hereto as **Exhibit A** pursuant to the requirements of 37 C.F.R. §1.121.

Accordingly, applicant respectfully requests that this Amendment be entered. After entry of this Amendment claims 1, 2, 7, 9-13, 17, 19, 22-24 and 27-38 will be pending and under examination upon entry of this Amendment.

In view of the remarks below, applicants maintain that the Examiner's rejections have been overcome, and respectfully request that they be withdrawn.

Claim Rejections Under 35 U.S.C. §112 (Second Paragraph)

The Examiner stated that claim 27 is rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention. The Examiner stated that the phrase "which polypeptide comprises consecutive amino acids having the sequence shown in SEQ ID NO:1" in claim 27 is vague and renders the claim indefinite. The Examiner stated that it is unclear what polypeptide is being referred to: the deletion mutant, substitution mutant, insertion mutant, or CD39 polypeptide.

In response, without conceding the correctness of the Examiner's position, and in order to expedite prosecution, applicants have amended claim 27. Claim 27 now recites, in part, "...administering to the human subject an effective amount of a deletion mutant, substitution mutant, or insertion mutant of the CD39 polypeptide, which *CD39* polypeptide comprises

consecutive amino acids having the sequence shown in SEQ ID NO:1 …" (emphasis added). Applicants point out that the polypeptide referred to in amended claim 27 is the CD39 polypeptide. Therefore, applicants maintain that the language of amended claim 27 is clear and definite.

In view of the above remarks, applicants maintain that amended claim 27 satisfies the requirements of 35 U.S.C. §112, second paragraph and respectfully requests that the Examiner reconsider and withdraw this ground of rejection.

Claim Rejections Under 35 U.S.C. §112 (First Paragraph)

The Examiner stated that claims 17-20 and 22-24 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The Examiner stated that claims 17-20 and 22-24 are directed to a method for determining whether a compound which increases ADP catabolism inhibits platelet aggregation or leucocyte accumulation and does not increase intracerebral hemorrhage (ICH) for treating or preventing thrombotic or ischemic disorder in a subject by using an animal model and measuring the stroke outcome and the incidence of ICH, and comparing the stroke outcome and incidence of ICH with or without a test compound. The Examiner further stated that the method of determining whether a compound does not increase ICH by measuring the incidence of ICH and comparing the incidence of ICH with or without the test compound is considered new matter because the specification fails to provide support for such method. The Examiner also stated that page 14, lines 19-32, of the specification cited in the amendment filed 7-29-02 only discloses measuring stroke outcome, platelet and/or fibrin

deposition and comparing the results but fails to support the claimed method.

In response, applicants respectfully traverse the Examiner's rejection.

Applicants maintain that all elements of claims 17-20 and 22-24 except whether a compound does or does not increase intracerebral hemorrhage (ICH) as compared with the incidence of ICH in the absence of the test compound can be found in the specification at page 14, lines 19-32. Support for the missing element is on page 23, lines 7-15 when read together with page 27 lines 19-31; page 29 lines 34 and 35; and page 30, lines 1-7.

In view of the above remarks, applicants maintain that claims 17-20 and 22-24 satisfy the written description requirement of 35 U.S.C. §112, first paragraph and respectfully request that the Examiner reconsider and withdraw this ground of rejection.

Claim Rejections Under 35 U.S.C. §112 (First Paragraph)

The Examiner further stated that claims 1-13, 16 and 27 are rejected under 35 U.S.C. §112, first paragraph, because the specification, while being enabling for the use of soluble CD39 in the treatment and prevention of thrombotic and ischemic disorders in mice and for the use of BIBU52 in rhesus and marmoset monkeys (Guth et al., abstract), does not reasonably provide enablement for the use of a CD39 polypeptide for treating or preventing stroke in a human subject, or for the use of an active fragment comprising amino acids 1-50 of SEQ ID NO:2 or about 20-80 amino acids of SEQ ID NO:1 that mimics the active site, or for the use of any deletion mutant, insertion mutant, or any truncated mutant of CD39 polypeptide for treating or preventing stroke in a human subject. The Examiner stated that the specification does not enable any person skilled in the art to which it pertains, or

with which it is most nearly, to make and/or use the invention commensurate in scope with these claims.

The Examiner stated that claims 1-13, 16 and 27 are directed to a method for treating or preventing stroke in a human subject comprising administering to the human subject a CD39 polypeptide comprising SEQ ID NO:1 or an active polypeptide fragment thereof, an active fragment comprising amino acid 1-50 of SEQ ID NO:2 or about 20-80 amino acid of SEQ ID NO:1 that mimics the active site, or a deletion mutant, insertion mutant, or a truncated mutant of CD39 polypeptide to inhibit ADP-mediated platelet aggregation by increasing ADP catabolism without increasing incidence of ICH in the human subject. The Examiner further stated that the claims encompass using a CD39 polypeptide, any active fragment comprising amino acid 1-50 of SEQ ID NO:2 or about 20-80 amino acid of SEQ ID NO:1 that mimics the active site, or any deletion mutant, insertion mutant, or a truncated mutant of CD39 polypeptide to treat or prevent stroke in a human subject. The Examiner stated that the specification only discloses the use of soluble CD39 in the treatment and prevention of thrombotic and ischemic disorders in mice. The Examiner stated that Guth discloses a non-peptide molecule, BIBU52, that can inhibit the aggregation of human platelets in platelet-rich plasma induced by collagen, ADP, and a thrombin-receptor activating peptide. The Examiner stated that BIBU 52 inhibits aggregation in plasma from rhesus and marmoset monkeys but no in rat plasma (e.g. abstract). The Examiner stated that the specification fails to provide adequate guidance and evidence whether and how a CD39 polypeptide comprising the sequencing of SEQ ID NO:1 or active fragment thereof, or active fragment comprising amino acid 1-50 of SEQ ID NO:2 or about 20-80 amino acid of SEQ ID NO:1 that mimics the active site, any deletion mutant, insertion mutant, or any truncated mutant of CD39 polypeptide can be used to treat or prevent stroke in a human subject and does not increase incidence of ICH.

The Examiner stated that Gura (Science, Vol. 278, p. 1041-1042, 1997) reports "The fundamental problem in drug discovery for cancer is that the (animal) model systems are not predictive at all" and "The animals apparently do no handle the drugs exactly the way the human body does. And attempts to use human cells in culture do not seem to be faring any better, partly because cell culture provides no information about whether a drug will make it to the tumor site" (e.g. p. 1041, first column). The Examiner stated that, similarly, the effect of a CD39 polypeptide comprising SEQ ID NO:1 or its active fragment thereof in treating or preventing stroke in a mouse model does not necessarily mean that said CD39 polypeptide could be used to treat or prevent stroke in a human subject and does not increase incidence of ICH. The Examiner stated that the specification and the teachings of the prior art only disclose the effect of CD39 polypeptide in vitro or in mouse model. The Examiner stated that there is no evidence or record that a CD39 polypeptide comprising the sequence of SEQ ID NO:1 or active fragment thereof, or active fragment comprising amino acid 1-50 of SEQ ID NO:2 or about 20-80 amino acid of SEQ ID NO:1 any deletion mutant, insertion mutant, or any truncated mutant of CD39 polypeptide can be used to treat or prevent stroke in a human subject and does not increase incidence of ICH. The Examiner stated that the claims specify treating or preventing stroke in a human subject but the specification fails to provide sufficient enabling disclosure to enable the claimed method. The Examiner also stated one can not extrapolate the therapeutic effect in an animal model, such as a mouse model, to the success in treating or preventing stroke in a human subject and, therefore, one skilled in the art at the time of the invention would have to engage in undue experimentation to practice over the full scope of the invention claimed.

The Examiner stated that in addition, the specification fails to provide adequate guidance and evidence whether and how an active fragment comprising amino acid 1-50 of SEQ ID NO:2 or

about 20-80 amino acid of SEQ ID NO:1 that mimics the active site (claims 6 and 7), any deletion mutant, insertion mutant, or any truncated mutant of CD39 polypeptide (claim 27) can be used to treat or prevent stroke in a human subject and does not increase incidence of ICH. The Examiner stated that the specification only discloses using a soluble CD39 polypeptide in treating or preventing stroke in mice and the cited reference, i.e. Shulte et al., 1999, discloses that apyrase conserved regions (ACR)-1, -4, and -5 within CD39 polypeptide are required for maintenance of biochemical activity of the CD39 polypeptide (e.g. abstract). The Examiner further stated that therefore, a CD39 polypeptide mutant must comprise ACR-1, -4, and -5 in order to maintain its biochemical activity so as to treat or prevent stroke in mice. The Examiner stated that the claims encompass using any CD39 polypeptide mutant comprising only one or two ACRs to treat or prevent stroke in a human subject and does not increase incidence of ICH. The Examiner also stated that there is also no evidence of record that adding additional amino acid residues between ACRs within CD39 polypeptide would not affect the biochemical activity of the CD39 polypeptide for treating or preventing stroke in a human subject and does not increase incidence of ICH.

The Examiner stated that further, the amino acid sequence of a protein determines its structural and functional properties, and predictability of which amino acids can be removed from a protein's sequence and still result in similar activity is extremely complex, and well outside the realm of routine experimentation, because accurate predictions of a protein's structure from mere sequence data are limited. The Examiner stated that Rudinger, 1976 (Peptide Hormones, Edited by Parsons, University Park Press, Baltimore, p. 1-7), points out that "The significance of particular amino acids and sequences for different aspects of biological activity cannot be predicted a priori but must be determined from case to case by painstaking experimental study" (e.g.p.6). Kaye et al. 1990 (Proc. Natl. Acad. Sci. USA, Vol. 87, pp. 6922-6926) teaches

that "A single amino acid substitution results in a retinoblastoma protein defective in phosphorylation and oncoprotein binding" (e.g. Title). The Examiner stated that Skolnick et al., 2000 (Trends in Biotech, Vol. 18, p. 34-39) states "Sequence-based methods for function prediction are inadequate because of the multifunctional nature of proteins. The Examiner stated that however, just knowing the structure of the protein is also insufficient for prediction of multiple functional sites. The Examiner stated that structural descriptors for protein functional sites are crucial for unlocking the secrets in both the sequence and structural-genomics projects" (e.g. abstract). The Examiner stated that Skolnick further states that "Knowing a protein's structure does not necessarily tell you its function" and "Because proteins can have similar folds but different functions, determining the structure of a protein may or may not tell you something about is function" (e.g. p36, box 2). The Examiner stated that in view of the lack of evidence that a CD39 polypeptide or its various mutants can be used to treat or prevent stroke in a human subject and does not increase incidence of ICH and the unpredictability of a protein function from mere amino acid sequences, one skilled in the art at the time of the invention would not know how to use the claimed CD39 and its variants for the claimed method.

The Examiner stated that for the reasons discussed above, it would have required undue experimentation for one skilled in the art at the time of the invention to practice over the full scope of the invention claimed. The Examiner stated that this is particularly true given the nature of the invention, the state of the prior art, the breadth of the claims, the amount of experimentation necessary, the working examples given and scarcity of guidance in the specification of guidance in the specification, and the unpredictable nature of the art.

In response, applicants traverse the Examiner's rejection.

In response to the Examiner's statement that the specification "does not reasonably provide enablement for the use of CD39 polypeptide for treating or preventing stroke in a human subject, or for the use of an active fragment comprising amino acid 1-50 of SEQ ID NO. 2 or about 20-80 amino acid of SEQ ID NO. 1 that mimics the active site, or for the use of any deletion mutant, insertion mutant, or any truncated mutant of CD39 polypeptide for treating or preventing stroke in a human subject", the applicants set forth the following remarks. The Examiner alleges that a CD39 polypeptide mutant must comprise ACR-1, -4 and -5 in order to maintain its biochemical activity so as to treat or prevent stroke in mice and alleges that support for these requirements can be found in the Schulte et al., 1999 reference (previously of record).

The applicants contend that the teachings of Schulte et al. are not relevant to that which is claimed. Applicants note that the nowhere in the claims do the applicants recite the use of a CD39 variant which contain either a FLAG-tag or a GPI anchor.

Applicants contend that at the time of the invention, methods of conveniently synthesizing polypeptide fragments were well known in the art and that identification of therapeutically promising fragments using the methods for screening compounds disclosed in the specification would not have required undue experimentation.

With respect to the Examiner's statement that "one cannot extrapolate the therapeutic effect in an animal model, such as a mouse model, to the success in treating or preventing stroke in a human subject" applicants set forth the following remarks. Applicants note that the Examiner cites Gura, which discusses the problems of animal models in cancer therapy. Applicants contend that cancer therapy is not stroke therapy, and that the arguments made by Gura are not readily applied to stroke therapy.

Applicants also note that those skilled in the art routinely use murine models of stroke as a predictor of success in human stroke therapy (see Jones et al., Annals of the New York Academy of Science, Volume 825, pages 281-287, October 15, 1997; **Exhibit B**). Applicants direct the Examiner to the statement by Jonas et al. (page 285) that "the corrections between laboratory and clinical results for five putatively neuroprotective treatments are compatible with the view that the influence of such a treatment on infarct size in animals during a given time window predicts human functional outcome responses to this treatment in the same time window..." Applicants maintain that it was known in the art, at the time of the invention that an animal stroke model can be used to predict the success of a potential therapeutic compound in treating or preventing stroke in a human subject.

Furthermore, only a reasonable correlation between animal *in vivo* examples and claimed method of use are expected for the animal *in vivo* example to be considered a working example (M.P.E.P. §2164.02). Indeed, if the art recognizes a particular model as correlating to a specific condition then it should be accepted as such unless the Examiner has evidence it does not correlate. Examiner has provided no such evidence. Applicants note that thrombolysis murine stroke models have been predictive of clinical results. Kilic et al. (Neuroreport, volume 10, pages 107-111, January 18, 1999; **Exhibit C**), used a mouse model of stroke to test whether tPA administration improves brain recovery after transient vascular occlusion. Kilic et al. found that [t]reatment with recombinant tPA after reversible thread occlusion of the middle cerebral artery in mice accelerates blood recirculation, reduces infarct volume and brain swelling and improves neurological performance (page 110, second column, last paragraph through page 111, first column, first paragraph).

The efficacy of tPA as a treatment for stroke observed by Kilic et al. using a murine model of stroke correlates well with efficacy of tPA for the treatment of stroke observed in a clinical trial conducted by the National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group (NINDS tPA trial) (New England Journal of Medicine, volume 333, number 24, pages 1581-1587; **Exhibit D**). The NINDS tPA trial concluded that "despite an increased incidence of intracerebral hemorrhage, an improvement in clinical outcome at three months was found in patients treated with intravenous t-PA within three hours of the onset of acute ischemic stroke (p.1586, second column, last full paragraph).

Using a murine stroke model, CD39 was shown by the applicants in the specification to (1) increase cerebral cortical blood flow (p. 27, lines 4-7 and Figure 3A) (2) decrease infarct volume (p.27, lines 10-11 and Figure 3B), (3) decrease the severity of neurological deficit (p. 27, lines 14-16 and Figure 3C), and (4) decrease incidence of mortality (page 27, lines 14-16 and figure 3D). Applicants contend that the specification provides support for the efficacy of CD38 in improving aspects of stroke pathophysiology known to be relevant in human stroke.

Applicants further note that a rigorous or an invariable correlation is not required (M.P.E.P. §2164.02). Applicants maintain, therefore, in view of the use of animal stroke models in the art, the success of animal stroke models, the examples in the specification, and the good degree of correlation present, that the specification is fully enabling for the claimed invention.

In view of the above remarks, applicants maintain that claims 1-13, 16 and 27 satisfy the enablement requirement of 35 U.S.C. §112, first paragraph and respectfully requests that the Examiner reconsider and withdraw this ground of rejection.

Conclusion

Applicants maintain that pending claims 1, 2, 7, 9-13, 17-19, 22-24 and 27-38 are in condition for allowance, and respectfully request allowance of these claims.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicants' undersigned attorney invite the Examiner to telephone him at the number provided below.

No fee, other then the enclosed amount of $543.00, is deemed necessary in connection with the filing of this Amendment. If any other fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 03-3125.

Respectfully submitted,

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I hereby certify that this correspondence is being deposited this date with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to:

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John P. White Date
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